Supporting Text

Mutagenesis. The *A. aeolicus lpxC* gene encoding a single amino acid substitution (A or G) for C193 was prepared from the plasmid pAaLpxC (pET21a) containing the cloned *lpxC* gene (ref. 1; the numbering scheme used for *E. coli* LpxC is adapted for *A. aeolicus* LpxC). These amino acid substitutions were chosen based on sequence alignments revealing A or G in the comparable position (C193 is not conserved) in other LpxC isoforms. The protocol outlined in the QuikChange site-directed mutagenesis kit (Stratagene) was followed using primers (29–37 nucleotides) replacing the codon for the nonconserved C193 (TGC) with that for A (GCC) or G (GGC). The resulting plasmids containing each mutation were transformed into XL-1 Blue supercompetent cells (Stratagene) and colonies selected by ampicillin resistance. After isolation of each plasmid, the *lpxC* gene sequence was analyzed by the University of Michigan DNA Sequencing Core. On ensuring that only the desired mutation was incorporated, the DNA was transformed into *E. coli* strain BL21(DE3)pLysS for overexpression. Expression levels of the mutants were tested by growing a single colony in 5-ml cultures of LB broth containing 100 μg/ml ampicillin and 30 μg/ml chloramphenicol. These cultures were grown at 34°C until they reached OD_{600} = 0.5, whereupon protein expression was induced by the addition of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) and 100 μM $ZnSO_4$. The cultures were incubated at 34°C for 5 h and harvested by centrifugation. The cells were resuspended in 25 mM Hepes, pH 7, with 0.2 mg/ml lysozyme and 180 units of endonuclease (*Serratia marcescens* recombinant; Sigma), and incubated at room temperature for ≈30 min. Cell lysates were centrifuged to remove insoluble fractions. Soluble fractions were analyzed by SDS/PAGE (Bio-Rad 12% Tris•HCl Ready Gels) stained with Coomassie blue. *A. aeolicus* LpxC protein bands were clearly present at ≈32 kD (as compared to standard proteins). As the C193A mutant expressed at higher levels than C193G, C193A LpxC was selected for further manipulation.

Deletion mutants (ΔS290-L294, ΔD284-L294, and ΔK280-L294) of the *A. aeolicus lpxC* gene were prepared from the plasmid encoding C193A LpxC as described above. The Stratagene kit was employed using primers replacing amino acid coding sequences with

two sequential stop codons (TAA•TGA or TGA•TAA). Convenient insertions of stop codons resulted in deletions of 5, 11, or 15 aa from the C terminus of LpxC. Transformations, sequencing, and overexpression were carried out as described above.

The C193A/ΔD284-L294 LpxC variant was used for crystallographic experiments and this variant was purified as described (2) with an additional purification step. Following elution from the Reactive red 120 fast-flow column, fractions containing LpxC were pooled and concentrated at room temperature in an Amicon pressure cell over a YM10 membrane to a volume of ≈3.5 ml. This sample was then loaded onto a Superdex 200 column and eluted with 25 mM Hepes (pH 7.0)/50 mM NaCl/0.5 mM $ZnSO_4$. Finally, protein was concentrated to 2.2 mg/ml (Amicon, YM10) and used for crystallization experiments.

Calorimetry. A representative titration curve from an isothermal titration calorimetry experiment with *A. aeolicus* LpxC and lauric acid is depicted in Fig. 6.

Organic Synthesis. ***Dodecane sulfonyl chloride.*** Dodecyl sulfonic acid salt (1 g, 3.68 mmol) was mixed with phosphorus pentachloride (1.53 g, 7.35 mmol) in a clean, dry flask. A reflux condenser was attached and the flask was heated in an oil bath at 150ºC for 30 min. The mixture was then warmed on a steam bath while stirring rapidly. The solution was filtered through dry filter paper, the filtrate was washed twice with 15 ml of water, and the ether was removed under a vacuum. The crude product required no further purification for the next step.

Dodecane sulfonamide. A suspension of dodecane sulfonyl chloride in anhydrous ether (30 ml) was stirred at room temperature and treated with ammonia (gas) for 45 min. The mixture was stirred for an additional 1 h. The solid sulfonamide was recovered by filtration and recrystallized from ethanol.

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.62 (s, 2H, $–NH_2$), 3.12 (t, 2H, CH_2-N), 1.64–1.94 (m, 4H), 1.40–1.20 (bs, 18H), 0.82 (t, 3H, $–CH_3$).

Analysis calculated for $C_{12}H_{27}O_2NS$: C, 57.83; H, 10.84; N, 5.62. Found: C, 57.13; H, 11.52; N, 5.64.

Diethyldodecylphosphate. Dodecylmagnesium bromide (1 M in diethyl ether, 2 ml, 4.0 mmol) was added to a mixture of diethyl chlorophosphate (2.07 g, 12 mmol) in 10 ml of tetrahydrofuran (THF) at –78ºC. After being stirred for 1 h, the mixture was allowed to warm to room temperature. The reaction was quenched with water and the solution was adjusted to a pH of 7 by the addition of 1 N HCl. Dilution with additional water was followed by extraction three times with diethyl ether. Concentration followed by silica gel column chromatography (hexane/ethyl acetate) gave the desired product.

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.92–4.15 (m, 4H), 1.52–1.75 (m, 2H), 1.18–1.38 (m, 26H), 0.78–0.90 (t, 3H).

Dodecane phosphonic acid. Bromotrimethylsilane (1.0 ml, 7.84 mmol) was added to a solution of diethyldodecylphosphate (0.3 g, 0.89 mmol) in CH_2Cl_2 (15 ml) at room temperature and the reaction was stirred for 3 days. The reaction was quenched by the addition of 1 ml of H_2O and the mixture was stirred for 20 min. The solution was concentrated and made basic by the addition of saturated $NaHCO_3$. The solution was then evaporated and the resulting solid was dissolved in a minimal amount of water. Concentrated HCl was added to acidify the solution. The white precipitate that formed was filtered and dried to give the desired product.

^{1}H NMR (500 MHz, CD_3OD) δ 1.54–1.70 (m, 4H), 1.39–1.28 (bs, 18H), 0.88 (t, 3H, –CH_3).

Analysis calculated for $C_{12}H_{27}O_3P$: C, 57.60; H, 10.80. Found: C, 57.29; H, 11.25.

Dodecylboronate pinanediol. To a solution of dodecene (1 g, 7.13 mmol) in THF (10 ml) was added BH_3•THF (1 M in THF, 14.2 ml, 14.2 mmol) at room temperature. The

reaction mixture was stirred overnight and the reaction was quenched by addition of methanol until bubbling ceased. The solvent was evaporated under vacuum and the residue was dissolved in CH_2Cl_2 (15 ml) at room temperature. (1*S*, 2*S*, 3*R*, 5*S*)-(+)-pinanediol (4.85 g, 28.5 mmol) was then added, and after stirring overnight, the solvent was concentrated. The boronic ester was purified by silica column chromatography (hexane/ethyl acetate).

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.26 (dd, 1H), 2.30–2.38 (m, 1H), 2.20 (m, 1H), 2.02 (m, 1H), 1.80–1.95 (m, 2H), 1.36–1.48 (m, 5H), 1.20–1.36 (brs, 22H), 0.98–0.92 (m, 9H).

Dodecane boronic acid. To a solution of the pinanediol ester (0.287 g, 0.86 mmol) in 8 ml was added BCl_3 (1 M in CH_2Cl_2, 3.45 ml, 3.45 mmol) at –78ºC. The reaction mixture was stirred for 1 h. The cooling bath was removed and the reaction was stirred for an additional 30 min. The reaction was quenched with H_2O (10 ml) and then stirred for another 20 min. The two phases were separated and the organic phase was washed with 10% acetic acid. The combined aqueous layers were then washed three times with diethyl ether. The aqueous layer evaporated slowly in the hood and the desired product was then recrystallized from THF/H_2O (2:1).

^{1}H NMR (500 MHz, CD_3OD) δ 1.23–1.35 (brs, 20H), 0.89 (t, 3H), 0.75 (t, 2H).

^{1}H B NMR: 31.75 ppm.

1. Jackman, J. E., Fierke, C. A., Tumey, L. N., Pirrung, M., Uchiyama, T., Tahir, S. H., Hindsgaul, O. & Raetz, C. R. H. (2000) *J. Biol. Chem.* **275,** 11002–11009.

2. Jackman, J. E., Raetz, C. R. H. & Fierke, C. A. (2001) *Biochemistry* **40,** 514–523.